Neuropeptide Y Inhibits Neurogenic Inflammation in Guinea-pig Airways

(1)

博士論文

Neuopeptide Y Inhibits Neurogenic Inflammation in Guinea-pig Airways

(ニューロペプタイドYのモルモット気道における神経原性炎症抑制効果の研究)

東北大学医学部

内科学第一講座

高橋　識至

博士論文

Neuopeptide Y Inhibits Neurogenic Inflammation in Guinea-pig Airways

（ニューロペプタイドYのモルモット気道における神経原性炎症抑制効果の研究）

東北大学医学部

内科学第一講座

高橋　識至

NEUROPEPTIDE Y INHIBITS NEUROGENIC INFLAMMATION IN GUINEA-PIG AIRWAYS.

First Department of Internal Medicine

Tohoku University School of Medicine

Sendai Japan

TEL: 022-274-1111 (EXT 2503)

FAX: 022-233-7985

Tsuneyuki Takahashi

ABSTRACT

I examined the effect of neuropeptide Y (NPY) on neurogenic airway microvascular leakage. Male Dunkin-Hartley guinea-pigs (250-350g) were anesthetized with urethane (2g/kg, ip). Atropine and propranolol (each 1mg/kg, iv) were administered 30 min before the experiment. After pretreatment with saline (vehicle for NPY) or NPY (1-100μg/kg, iv), Evans blue dye (EB, 30mg/kg, iv) was administered. Then, bilateral vagal nerves were electrically stimulated (5V, 7Hz, 5ms duration, for 3min) to induce airway plasma leakage. Airways were divided into 4 sections (trachea; Tr, main bronchi; MB, central intrapulmonary airways; cIPA, peripheral IPA; pIPA), and incubated in formamide (37°C, 16hr). The concentration of EB dye was measured by a spectrophotometer. Furthermore, I examined the effect of NPY on exogenous substance P (SP, 0.3μg/kg, iv)-induced plasma extravasation. Bilateral vagal stimulation significantly increased the leakage of dye in Tr to pIPA. NPY did not affect basal leakage, but significantly inhibited neurogenic plasma extravasation in a dose dependent manner, with maximal inhibitions of 42.3 (Tr), 67.7 (MB), 38.2 (cIPA), and 26.3% (pIPA) at 30μg/kg. Exogenous SP-induced plasma extravasation was not inhibited by NPY. I conclude that NPY inhibits neurogenic inflammation by prejunctional inhibition of neuropeptide release from airway sensory nerve terminals.

Keywords: airway permeability, asthma, substance P,
prejunctional modulation, potassium channel.

Neurogenic inflammation, caused by the antidromic release of neuropeptides from sensory nerves via axon reflex mechanisms, is now considered to be an important pathological factor in inflammatory airway diseases including bronchial asthma (Barnes 1986) (Table 1). Recent advances in pharmacology and physiology have revealed that the neuropeptides released from sensory nerves mimic many of the pathophysiological features of asthma, such as bronchoconstriction, airway plasma leakage, mucus secretion and vasodilation (Barnes 1989; Barnes et al. 1991). Therefore, in addition to the classic role of cholinergic and adrenergic innervation of airways, the sensory nerves may have an important role in the pathogenesis of asthma. Actually, tachykinins, especially substance P (SP), cause airway plasma leakage which is a common pathological change observed in asthmatic airways. Recently, we demonstrated that pretreatment with tachykinin receptor antagonist protects against bradykinin-induced bronchoconstriction and coughing in asthmatic patients (Ichinose et al. 1992), and reduced cough and sputum in chronic bronchitis patients (Ichinose et al. 1993). Therefore, the modulation of neurogenic inflammation through neuropeptide release from sensory nerve endings may be a useful approach in controlling these diseases.

There are several ways to modulate neurogenic inflammation (Figure 1): 1) reduction of sensory nerve stimulation, 2) presynaptic inhibition of neuropeptide release, 3) blockade of tachykinin receptors by NKs antagonists at the effected organs. Among them, presynaptic modulation appears to be useful way to

modulate neurogenic inflammation. It has been reported that several different agonists, such as μ-opioid (Barthó and Szolcsányi 1981; Barthó et al. 1982; Belvisi et al. 1989; Rogers and Barnes 1989), α_2-agonist (Grundström and Andersson 1985; Matran et al. 1989), ɣ-aminobutylic $acid_B$ ($GABA_B$) (Belvisi et al. 1989), histamine H_3 (Ichinose et al. 1989, 1990), corticotropin-releasing factor (Wei et al. 1987, 1989) and galanin (Giuiliani et al. 1989), inhibit sensory nerve-mediated bronchoconstriction by modulating neuropeptide release at sensory nerve terminals (Figure 2). The K^+-channel opener cromakalim is also effective in inhibiting sensory nerve-mediated bronchoconstriction in guinea-pigs presynaptically (Ichinose et al. 1989), indicating that K^+-channel activation also has inhibitory effect on neurogenic inflammation at nerve endings.

Neuropeptide Y (NPY) is a 36 amino acid peptide which was first isolated from porcine brain (Tatemoto 1982; Tatemoto et al. 1982), and found in many central and peripheral neurons in several mammalians including human. It is known that there are 3 different structural types of mammalian NPY and that the human type is the same as guinea-pig, rat and rabbit (Minth et al. 1984; O'Hare et al. 1987; Sillard et al. 1989)(Figure 3). In airways, NPY has been shown to present together with noradrenaline in sympathetic nerves (Sheppard et al. 1984). It has been reported that NPY modulates the cholinergic bronchoconstrictor response by reducing acetylcholine release from nerve terminals (Stretton and Barnes 1988). Furthermore, NPY inhibits sensory nerve-mediated bronchoconstriction via the reduction of neuropeptide release from sensory nerve terminals

(Grundemar et al. 1990; Stretton et al. 1990). However, the effect of NPY on sensory nerve-mediated plasma leakage in the airways has not yet been elucidated (Figure 4). Since NPY has a potent vasoconstrictor action (Wahlestedt et al. 1986; Salonen et al. 1988; Lacroix 1989), the effect of NPY on airway microvascular leakage may be complicated. Therefore, the aim of this study is to demonstrate the effect of NPY on airway plasma leakage in guinea-pig airways. I evoked neurogenic inflammation by vagal nerve stimulation after muscarinic and β-adrenergic blockage, and I quantified airway plasma leakage using the Evans blue dye technique (Rogers et al. 1989; Udaka et al. 1970). In addition, to determine whether NPY acts on nerve endings (prejunctional) or leaky sites (postjunctional), I examined the effect of NPY on exogenously applied SP-induced plasma leakage, because it is known that SP is the main neurotransmitter which induces airway plasma leakage (Figure 5).

METHODS

Animal preparation

Male Dunkin-Hartley guinea-pigs (Funabashi Farm, Hamamatsu, Japan) weighing 250-350 g were anesthetized by intraperitoneal injection of urethane (2 g/kg). The carotid artery and jugular vein were cannulated to monitor systemic blood pressure and to inject drugs, respectively. All animals were pretreated 30 min before experimentation with atropine and propranolol (each 1 mg/kg iv) to exclude the effect of muscarinic and β-adrenergic receptors, respectively. The dose of atropine and propranolol was determined according to previous studies (Belvisi et al. 1989; Ichinose et al. 1990).

Protocol

Both cervical vagus nerves were carefully dissected free and sectioned at the level of the fifth tracheal cartilage ring, and their peripheral ends were placed on platinum electrodes. I studied the effect of NPY on sensory nerve-mediated Evans blue dye leakage in 6 groups: *group 1* saline (1 ml/kg, iv) and sham nerve stimulation (n=6); *group 2*, NPY (30 μg/kg, iv) and sham nerve stimulation (n=6); *group 3*, saline and nerve stimulation (n=6); *group 4*, NPY (10 μg/kg, iv) and nerve stimulation (n=6); *group 5*, NPY (30 μg/kg, iv) and nerve stimulation (n=6); *group 6*, NPY (100 μg/kg, iv) and nerve stimulation (n=6). Saline or NPY

was administered 1 min before Evans blue dye injection (30 mg/kg, iv). Nerve or sham stimulation followed 1 min after injection of Evans blue dye. Nerves were stimulated by an electrical stimulator (SEN 3201, Nihon-Kohden, Japan) with parameters that have previously been found to be optimal under these conditions (5 V, 7 Hz, 5 ms pulse duration, for 3 min)(Belvisi et al. 1989; Ichinose et al. 1990).

The effect of NPY (30 µg/kg, iv) in the presence of the ATP-sensitive potassium channel blocker, glibenclamide (25 mg/kg, iv, n=6), or a vehicle for glibenclamide (glucose, NaOH, and H_2O, pH=10.8, n=6) was also examined. Glibenclamide or the vehicle for glibenclamide was injected slowly (over 4-5 min) 15 min before NPY administration. The dose of glibenclamide was chosen according to a previous study (Ichinose and Barnes 1989).

The effect of saline (n=6) or NPY (30 µg/kg, iv, n=6) on microvascular leakage evoked by exogenous SP (1 µg/kg, iv) was also studied in separate experiments. The dose of SP was chosen to give an increase in Evans blue dye extravasation which was comparable to that produced by vagus nerve stimulation. NPY or saline was injected followed 1 min later by Evans blue dye and by saline or SP after a further minute.

Vascular permeability quantitative analysis

Vascular permeability was quantified by the extravasation of Evans blue dye, which correlates well with extravasation of radiological albumin in the skin (Udaka et al. 1970) and airways

(Rogers et al. 1989).

The tissue content of Evans blue dye after experimental intervention was determined by perfusing the systemic circulation with saline to remove intravascular dye (Rogers et al. 1989). After induction of leakage (1 min after the completion of nerve stimulation), the thorax was opened and a blunt-ended, 13-gauge needle was passed through a left ventriculotomy into the aorta. The ventricles were cross-clamped and blood was expelled through an incision in the right atrium with 100 ml saline (pH 5.5) at 80 mmHg pressure until the perfusate was clear. The lungs were then removed. The connective tissues, vasculature, and parenchyma were gently removed. The main bronchi (MB) were separated from the trachea (Tr), and the remaining intrapulmonary airways were separated into central (cIPA, the proximal 3 mm portion) and peripheral (pIPA, the remaining distal portion). The tissues were blotted dry and incubated in formamide (37°C, 16 hr). The concentration of Evans blue dye was quantified from light absorbance at 620 nm (Spectrophotometer 220A, HITACHI, Tokyo, Japan) and its tissue content (ng dye/mg wet weight tissue) was calculated from a standard curve of dye concentrations in the range of 0.5 to 10 μg/ml.

Drugs

The following drugs were used: Evans blue dye, formamide, urethane, and glibenclamide (Sigma Chemical, St. Louis, MO, USA); NPY and SP (Peptide Institute Inc., Osaka, Japan); propranolol hydrochloride (Imperial Chemical Industries, Osaka, Japan);

atropine sulfate (Tanabe Pharmaceutical, Osaka, Japan). SP was prepared in stock solutions of 1 mg in 1 ml saline and stored at -20°C. Glibenclamide was sonicated in NaOH (0.1 M) at the concentration of 25 mg/ml, then diluted 1 in 4 with 5 % glucose (pH=10.8). All drug solutions were freshly prepared in saline on each day of experimentation. Evans blue dye (30 mg/ml in saline) was filtered through an Acrodisc membrane of 0.2 μm pore diameter.

Statistical analysis

Data are expressed as means ± SE. Because the distribution of data for the concentration of extractable Evans blue dye approximated a normal distribution but showed a positive skew, the Mann-Whitney *U* test (two-tailed) was used to test the null hypothesis. Mean arterial blood pressure (BP) was calculated from recorded traces as diastolic pressure + 0.33 x (systolic pressure - diastolic pressure). Data for mean BP approximated a Gaussian distribution, and Student's *t* test for paired or unpaired data (two-tailed) was used to compare mean values. Probability values of $p < 0.05$ were considered significant.

RESULTS

Bilateral vagal stimulation increased Evans blue dye extravasation in Tr, MB, cIPA, and pIPA, 3.6 ± 3.0 to 39.2 ± 3.2, 4.9 ± 4.2 to 94.0 ± 18.0, 7.7 ± 3.9 to 46.0 ± 3.6, 7.7 ± 4.2 to 42.6 ± 4.0 ng/ mg tissue (mean ± SE), respectively. NPY (30μg/kg, iv) alone had no significant effect on Evans blue dye extravasation but significantly inhibited the neurogenic plasma leakage in a dose-dependent manner (Figure 6, 7). At 30 μg/kg, the inhibitory effect of NPY was maximal, and Evans blue extravasation was reduced in Tr from 39.2 ± 3.2 to 22.6 ± 3.2 (42.3% inhibition, $p< 0.05$), in MB from 94.0 ± 18.0 to 34.0 ± 10.2 (63.8% inhibition, $p< 0.01$), in cIPA from 46.0 ± 3.6 to 27.1 ± 2.4 (41.1% inhibition, $p< 0.01$) and in pIPA from 42.6 ± 4.0 to 31.4 ± 1.0 ng/mg tissue (26.3% inhibition, $p< 0.05$).

Exogenous SP (0.3 μg/kg, iv) increased Evans blue dye leakage to an extent comparable to bilateral vagal stimulation in all airway tissues. However, NPY (30 μg/kg, iv) had no inhibitory effect on exogenous SP-mediated airway microvascular leakage in any airway tissues (Figure 8).

The inhibitory effect of NPY (30 μg/kg, iv) was not affected by pretreatment with glibenclamide (25 mg/kg, iv) in any airway tissues (Figure 9, 10).

NPY significantly increased mean blood pressure dose-dependently (Table 2).

DISCUSSION

The present data show that NPY inhibits neurogenic plasma leakage induced by vagal nerve stimulation in guinea-pig airways. Because exogenous SP-induced Evans blue extravasation was not reduced by NPY, I concluded that NPY inhibits neuropeptide release from sensory nerve terminals. Furthermore, the inhibitory effect of NPY was not altered by pretreatment with the ATP sensitive potassium channel blocker, glibenclamide, suggesting that this channel is not involved in the modulatory action of NPY.

Evans blue dye has been shown to combine quantitatively with plasma proteins in vivo (Udaka et al. 1970) and correlates well with extravasation of radiolabeled albumin in the airways (Rogers et al. 1989). The site of airway microvascular leakage appears to be the postcapillary venules, of which the endothelial cells were contracted in response to spasmogens, resulting in the formation of intracellular gaps (Persson 1987). This is supported by ultrastructual, pathophysiological, and pharmacological studies (Persson 1988).

It has been thought that the excitatory nonadrenergic noncholinergic (e-NANC) component of vagal stimulation is important in the mechanism of neurogenic inflammation in guinea-pig airways, since pretreatment with atropine, propranolol, phentolamine or hexamethonium do not affect the leakage (Ichinose et al. 1990; Lundberg and Saria 1982; Persson 1988). Furthermore, most of the vagally induced plasma leakage in airway

is reduced with capsaicin pretreatment (Lundberg and Saria 1982) or administration of a tachykinin antagonist (Lundberg et al. 1983), suggesting that neurogenic inflammation is due to the release of peptides such as SP from sensory nerves (Figure 5).

NPY is distributed in adrenergic nerves and is predominant in the nasal vessels, bronchial vessels and glands, while less marked in the nervation of airway smooth muscle (Sheppard et al. 1984). It has been shown that NPY inhibits cholinergic bronchoconstriction via a modulatory effect on cholinergic neurotransmission of postganglionic cholinergic nerves (Stretton and Barnes 1988). Furthermore, NPY modulates sensory nerve-mediated bronchoconstriction through an inhibitory action on neuropeptide release from sensory nerve terminals (Grundemar et al. 1990; Stretton et al. 1990). However, the effect of NPY on neurogenic plasma leakage has not yet been elucidated (Figure 4). In this study, I demonstrated that NPY inhibits sensory nerve-mediated airway microvascular leakage. Because NPY had no inhibitory effect on comparable Evans blue dye leakage induced by exogenous SP, it is suggested that NPY suppressed the neuropeptide release from airway sensory nerves.

NPY has a potent vasoconstrictor effect (Wahlestedt et al. 1986; Salonen et al. 1988; Lacroix 1989). An alteration in bronchial blood flow may affect the delivery of blood to leaky sites, the perfused surface area, and the microvascular hydrostatic pressure (Persson 1987). It is theoretically possible that the inhibitory effect of NPY on neurogenic airway plasma leakage may be simply due to a reduction of bronchial blood flow, which has been shown to be an important factor in the

regulation of vascular permeability in the skin (Brain and Williams 1985). If so, NPY should reduce exogenous SP-induced airway microvascular leakage. However, in this study, NPY did not alter the exogenous SP-induced leakage. Therefore, it seems unlikely that the NPY-induced vasoconstrictor effect itself contributed to the inhibitory action of NPY in this study. A recent report shows that phenylephrine produces vasopressor action without affecting the airway microvascular leakage induced by vagal nerve stimulation (Kuo et al. 1992), indicating that vasopressor action itself does not simply modify airway neurogenic plasma leakage.

Several other agonists such as μ-opioid (Barthó and Szolcsányi 1981; Barthó et al. 1982; Belvisi et al. 1989; Rogers and Barnes 1989), α_2-agonists (Grundström and Andersson 1985; Matran et al. 1989), $GABA_B$ (Belvisi et al. 1989), histamine H_3 (Ichinose and Barnes 1989; Ichinose et al. 1990), corticotropin-releasing factor (Wei and Kiang 1987, 1989) and galanin (Giuiliani et al. 1989), have been shown to inhibit neuropeptide release from sensory nerves by activating prejunctional receptors (Figure 2). In the lateral parabrachial nucleus in rat brain slices, stimulation of μ-opioid, $GABA_B$ and M_2-muscarinic receptors increases membrane potassium conductance and thus inhibits neuronal activation through hyperpolarization (Christie and North 1988). An ATP-sensitive potassium channel activator, cromakalim, mimics the effect of agonists at presynaptic receptors (Ichinose and Barnes 1989), thus this channel may be a common pathway of the presynaptic modulation (Figure 2). In

this study, however, the ATP-sensitive potassium channel blocker, glibenclamide, did not alter the inhibitory effect of NPY, suggesting that this channel is not involved in the inhibitory effect of NPY. More recently, it has been reported that the large conductance Ca^{2+}-activated K^{+}-channel blocker, charybdotoxin, reduces the inhibitory effect of NPY on sensory nerve-mediated bronchoconstriction (Stretton et al. 1992). Therefore, this channel in the sensory nerve terminals may be involved in the NPY-induced modulation of neuropeptide release in this study (Figure 11).

Neurogenic inflammation, which involves the release of neuropeptides from sensory nerves, is well established in rodent airways (Barnes et al. 1991; Lundberg and Saria 1982; Lundberg et al. 1983), but until recently there has been no direct evidence that these mechanisms are involved in human airway disease, partly because no specific antagonists of these peptides have been available. Recently, we have demonstrated that pretreatment with tachykinin receptor antagonist protects substantially against bradykinin-induced bronchoconstriction and coughing in asthmatic patients (Ichinose et al. 1992). This observation suggests that released tachykinins from airway sensory nerves have effects on human airways, although tachykinin-induced plasma leakage in human airways has not yet been demonstrated.

In conclusion, NPY inhibits sensory nerve-mediated plasma leakage in guinea-pig airways, presumably reducing the release of neuropeptides from sensory nerve terminals. Because NPY localizes in airway sympathetic nerves which are closely associated with sensory nerves, NPY may act as a safety device to

prevent neurogenic airway inflammation.

ACKNOWLEDGEMENT

The writer thanks Drs. Masakazu Ichinose, Hideyuki Yamauchi, Motohiko Miura, Natsuko Kageyama, Jun Ishikawa, Hiroshi Inoue, Tamotsu Takishima and Kunio Shirato for their helpful suggestions and Mr. Brent Bell for reading the manuscript.

REFERENCES

1) Barnes, P.J. (1986) Asthma as an axon reflex. *Lancet* 1: 242-245.

2) Barnes, P.J. (1989) New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J. Allergy Clin. Immunol.* 83: 1013-1026.

3) Barnes, P.J., Baraniuk, J.N. & Belvisi, M.G. (1991) Neuropeptides in the respiratory tract. part I. *Am. Rev. Respir. Dis.* 144: 1187-1198.

4) Barnes, P.J., Baraniuk, J.N. & Belvisi M.G. (1991) Neuropeptides in the respiratory tract. part II. *Am. Rev. Respir. Dis.* 144: 1391-1399.

5) Barnes, P.J., Belvisi, M.G. & Rogers, D.F. (1990) Modulation of neurogenic inflammation: novel approaches to inflammatory disease. *Trends Pharmacol. Sci.* 11: 185-189.

6) Barthó, L. & Szolcsányi, J. (1981) Opiate agonists inhibit neurogenic plasma extravasation in the rat. *Eur. J. Pharmacol.* 73: 101-104.

7) Barthó, L., Sebok, B. & Szolcsányi J. (1982) Indirect evidence for the inhibition of enteric substance P neurons by opiate agonists but not by capsaicin. *Eur. J. Pharmacol.* 77: 273-279.

8) Belvisi, M.G., Ichinose, M. & Barnes P.J. (1989) Modulation of non-adrenergic, non-cholinergic neural bronchoconstriction in guinea-pig airways via $GABA_B$-receptors. *Br. J. Pharmacol.*

97: 1225-1231.

9) Belvisi, M.G., Rogers, D.F. & Barnes P.J. (1989) Neurogenic plasma extravasation: inhibition by morphine in guinea pig airways in vivo. *J. Appl. Physiol.* 66: 268-272.

10) Brain, S.D. & Williams, T.J. (1985) Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. *Br. J. Pharmacol.* 86: 855-860.

11) Christie, M.J., & North, R.A. (1988) Agonists at μ-opioid, m_2-muscarinic and $GABA_B$-receptors increase the same potassium conductance in rat lateral parabrachial neurones. *Br. J. Pharmacol.* 95: 896-902.

12) Giuiliani, S., Amann, r., Papini, A.M., Maggi, C.A. & Meli, A. (1989) Modulatory action of galanin on responses due to antidromic activation of peripheral terminals of capsaicin-sensitive sensory nerves. *Eur. J. Pharmacol.* 163: 91-96.

13) Grundemar, L., Grundström, N., Johansson, I.G.M., Andersson, R.G.G. & Hakanson R. (1990) Suppression by neuropeptide Y of capsaicin-sensitive sensory nerve-mediated contraction in guinea-pig airways. *Br. J. Pharmacol.* 99: 473-476.

14) Grundström, N. & Andersson, R.G.G. (1985) In vivo demonstration of alpha-2-adrenoceptor-mediated inhibition of the excitatory non-cholinergic neurotransmission. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 328: 236-240.

15) Ichinose, M. & Barnes, P.J. (1989) Histamine H_3-receptors modulate nonadrenergic noncholinergic neural bronchoconstriction in guinea-pig in vivo. *Eur. J. Pharmacol.* 174: 49-55.

16) Ichinose, M. & Barnes P.J. (1989) A potassium channel activator modulates both excitatory noncholinergic and cholinergic neurotransmission in guinea pig airways. *J. Pharmacol. Exp. Ther.* 252: 1207-1212.

17) Ichinose, M., Belvisi, M.G. & Barnes, P.J. (1990) Histamine H_3-receptors inhibit neurogenic microvascular leakage in airways. *J. appl. Physiol.* 68: 21-25.

18) Ichinose, M., Nakajima, N., Takahashi, T., Yamauchi, H., Inoue, H. & Takishima, T. (1992) Protection against bradykinin-induced bronchoconstriction in asthmatic patients by neurokinin receptor antagonist. *Lancet* 340: 1248-1251.

19) Ichinose, M., Katsumata, U., Kikuchi, R., Fukushima, T., Ishii, M., Inoue, C., Shirato, K. & Takishima, T. (1993) Effect of tachykinin receptor antagonist on chronic bronchitis patients. *Am. Rev. Respir. Dis.* 147: A318.

20) Kuo, H-P., Liu, S. & Barnes, P.J. (1992) The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. *Eu. J. Pharmacol.* 221: 385-388.

21) Lacroix, J.S. (1989) Adrenergic and non-adrenergic mechanisms in sympathetic vascular control of the nasal mucosa. *Acta. Physiol. Scand.* 136(Suppl581: 1-63).

22) Lembeck, F., Donnerer, J., Tsuchiya, M. & Nagahisa, A. (1992) The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation. *Br. J. Pharmacol.* 105: 527-530.

23) Lundberg, J.M. & Saria, A. (1982) Capsaicin-sensitive vagal neurons involved in the control of vascular permeability in

rat trachea. *Acta. Physiol. Scand.* 115: 521-523.

24) Lundberg, J.M., Saria, A., Brodin, E., Rossel, S. & Folkers, K. (1983) A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pig. *Proc. Natl. Acad. Sci.* 80: 1120-1124.

25) Matran, R., Martling, C.-R. & Lundberg, J.M. (1989) Inhibition of cholinergic and non-adrenergic, non-cholinergic bronchoconstriction in the guinea pig mediated by neuropeptide Y and α_2-adrenoceptors and opiate receptors. *Eur. J. Pharmacol.* 163: 15-23.

26) Minth, C.D., Broom, S.R., Polak, J.M. & Dixon J.E. (1984) Cloning, characterization, and DNA sequence of a human cDNA encoding neuropeptide tyrosine. *Proc. Natl. Acad. Sci. USA* 81: 4577-4581.

27) O'Hare, M.M.T., Tenmoku, S., Aakerlund, L., Hilsted, L., Johnsen, A. & Schwartz T.W. (1988) Neuropeptide Y in guinea pig, rabbit, rat and man. Identical amino acid sequence and oxidation of methionine-17. *Regul. Pept.* 20: 293-304.

28) Persson, C.G.A. (1986) Role of plasma exudation in asthmatic airways. *Lancet* 2: 1126-1129.

29) Persson, C.G.A. (1987) Leakage of macromolecules from the tracheobronchial microcirculation. *Am. Rev. Respir. Dis.* 135: S71-75.

30) Persson, C.G.A. (1988) Plasma exudation and asthma. *Lung* 166: 1-23.

31) Rogers, D.F., & Barnes, P.J. (1989) Opioid inhibition of neurally mediated mucus secretion in human bronchi. *Lancet*

1: 930-932.

32) Rogers, D.F., Boschetto, P. & Barnes, P.J. (1989) Plasma exudation: correlation between Evans blue dye and radiolabeled albumin in guinea pig airways in vivo. *J. Pharmacol. Methods*. 21: 309-315.

33) Salonen, R.O., Webber, S.E. & Widdicombe, J.G. (1988) Effects of neuropeptides and capsaicin on the canine tracheal vasculature *in vivo*. *Br. J. Pharmacol*. 95: 1262-1270.

34) Sheppard, M.N., Polak, J.M., Allen, J.M. & Bloom, S.R. (1984) Neuropeptide Y: a newly discovered peptide is present in the mammalian respiratory tract. *Thorax* 39: 326-330.

35) Sillard, R., Agerberth, B., Mutt, V. & Jörnvall, H. (1989) Sheep neuropeptide Y. A third structural type of a highly conserved peptide. *FEBS LETTERS* 258: 263-265.

36) Stretton, C.D. & Barnes, P.J. (1988) Modulation of cholinergic neurotransmission in guinea-pig trachea by neuropeptide Y. *Br. J. Pharmacol*. 93: 672-678.

37) Stretton, C.D., Belvisi, M.G. & Barnes P.J. (1990) Neuropeptide Y modulates NANC neural bronchoconstriction in vivo and in vitro. *Am. Rev. Respir. Dis*. 141: A663.

38) Stretton, C.D., Miura, M., Belvisi, M.G. & Barnes P.J. (1992) Calcium-activated potassium channels mediate prejunctional inhibition of peripheral sensory nerves. *Proc. Natl. Acad. Sci. USA* 89: 1325-1329.

39) Tatemoto, K. (1982) Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. USA* 79: 5485-5489.

40) Tatemoto, K., Carlquist, M. & Mutt V. (1982) Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296:659-662.

41) Udaka, K., Takeuchi, Y. & Movat, H.Z. (1970) Simple method for quantitation of enhanced vascular permeability. *Proc.Soc. Exp. Biol. Med.* 133: 1384-1387.

42) Wahlestedt, C., Yanaihara, N. and Hakanson, R. (1986) Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regul. Pept.* 13: 307-318.

43) Wei, E.T. & Kiang, J.C. (1989) Peptides of the corticoliberin superfamily attenuate thermal and neurogenic inflammation in rat pawskin. *Eur. J. Pharmacol.* 168: 81-86.

44) Wei, E.T. & Kiang, J.C. (1987) Inhibition of protein exudation from the trachea by corticotropin-releasing factor. *Eur. J. Pharmacol.* 140: 63-67.

FIGURE LEGENDS

Figure 1 Three possible ways to modulate neurogenic inflammation.

Figure 2 Schematic explanation of presynaptic modulation of sensory nerve-mediated bronchoconstriction. H_3, histamine H_3. $GABA_B$, γ-aminobutylic $acid_B$.

Figure 3 Comparison of the structure of NPYs from different mammalian species. The one-letter code of amino acids has been used. Human NPY has a Glu residue (E) in position 10 and a Met residue (M) in position 17.

Figure 4 Schematic explanation of the effect of NPY in airways. ACh, acetylcholine. SP, substance P.

Figure 5 Two possible sites (presynaptic or postsynaptic) of the modulatory action of agonists on sensory nerve-mediated plasma leakage induced by vagal stimulation (VS). SP, substance P.

Figure 6 Effects of NPY on sensory nerve-mediated plasma extravasation induced by vagal stimulation (VS) in trachea and main bronchi. Values are means ± SE of 6 animals. Significantly different from saline (vehicle for NPY) values: * $P< 0.05$, ** $P<0.01$ (2-tailed Mann-Whitney U test).

Figure 7 Effects of NPY on sensory nerve-mediated plasma extravasation induced by vagal stimulation (VS) in central and peripheral intrapulmonary airways (IPA). Values are means ± SE of 6 animals. Significantly different from saline values: * $P< 0.05$, ** $P< 0.01$ (2-tailed Mann-Whitney U test).

Figure 8 Values are means ± SE of 6 animals expressed as tissue content of Evans blue dye (ng/mg tissue) after intravenous injection of substance P (0.3 µg/kg) after saline (SP) and substance P after NPY (30 µg/kg)(SP + NPY). No significant differences were observed (2-tailed Mann-Whitney U test).

Figure 9 Inhibition of sensory nerve-mediated plasma extravasation by NPY (30 μg/kg iv). Effects of vehicle for glibenclamide (glucose, NaOH and H_2O) and glibenclamide (GB, 25 mg/kg iv) on inhibitory effect of NPY are shown. A: trachea. B: main bronchi. Data are means ± SE of 6 animals. Differences between means were analyzed by 2-tailed Mann-Whitney *U* test.

Figure 10 Inhibitory effect of NPY (30μg/kg iv) on sensory nerve-mediated Evans blue dye extravasation in airways. Effects of vehicle for GB and GB (25mg/kg iv) on inhibitory effect of NPY are shown. A: central IPA. B: peripheral IPA. Data are means ±SE of 6 animals. Differences between means were analyzed by 2-tailed Mann-Whitney *U* test.

Figure 11 Schematic explanation of possible mechanism of NPY-induced presynaptic modulation of neurogenic inflammation. SP, substance P.

Table 1. *NEUROGENIC INFLAMMATION*

I) TACHYKININS CAUSE MANY OF THE PATHOPHYSIOLOGICAL FEATURES OF ASTHMA

- Bronchoconstriction
- Airway plasma leakage
- Mucus secretion
- Coughing
- Facilitation of cholinergic neurotransmission
- Airway hyperresponsiveness ?
- Tissue remodelling ?

II) SENSORY NERVE STIMULATION

- Bradykinin
- Leukotriene D_4
- Histamine
- Cold air
- Cigarette smoke
- Hypertonic saline

III) SIGNIFICANCE IN HUMAN AIRWAY DISEASES

- Tachykinin receptor antagonist is effective in the patients of asthma and chronic bronchitis.

IV) PROTECTION AGAINST THE EFFECT OF TACHYKININS

- Neuromodulation
- Tachykinin receptor antagonist

Table 2. Mean blood pressure changes after NPY (10 - 100 µg/kg, iv)

	Baseline (Torr)	NPY (Torr)	Δ Change (Torr)	% Change
NPY 10 µg/kg (n=6)	51.1 ± 3.4	73.0 ± 6.6*	21.8 ± 3.7	41.9 ± 5.3
NPY 30 µg/kg (n=6)	48.9 ± 5.7	78.4 ± 6.8*	29.8 ± 9.8	65.2 ± 28.1
NPY 100 µg/kg (n=6)	47.5 ± 2.9	87.3 ± 8.7**	39.8 ± 8.4	84.9 ± 20.8
Glibenclamide + NPY 30 µg/kg (n=6)	66.3 ± 2.8	96.0 ± 6.3**	31.4 ± 5.7	51.6 ± 11.2

Values are means ± SE. Δ Changes are defined as baseline value - neuropeptide Y (NPY) value. % Changes are defined as Δ Change/baseline value x 100. * $p < 0.05$, ** $p < 0.01$ compared with baseline values (Student´s paired *t* test).

Modulation of neurogenic inflammation

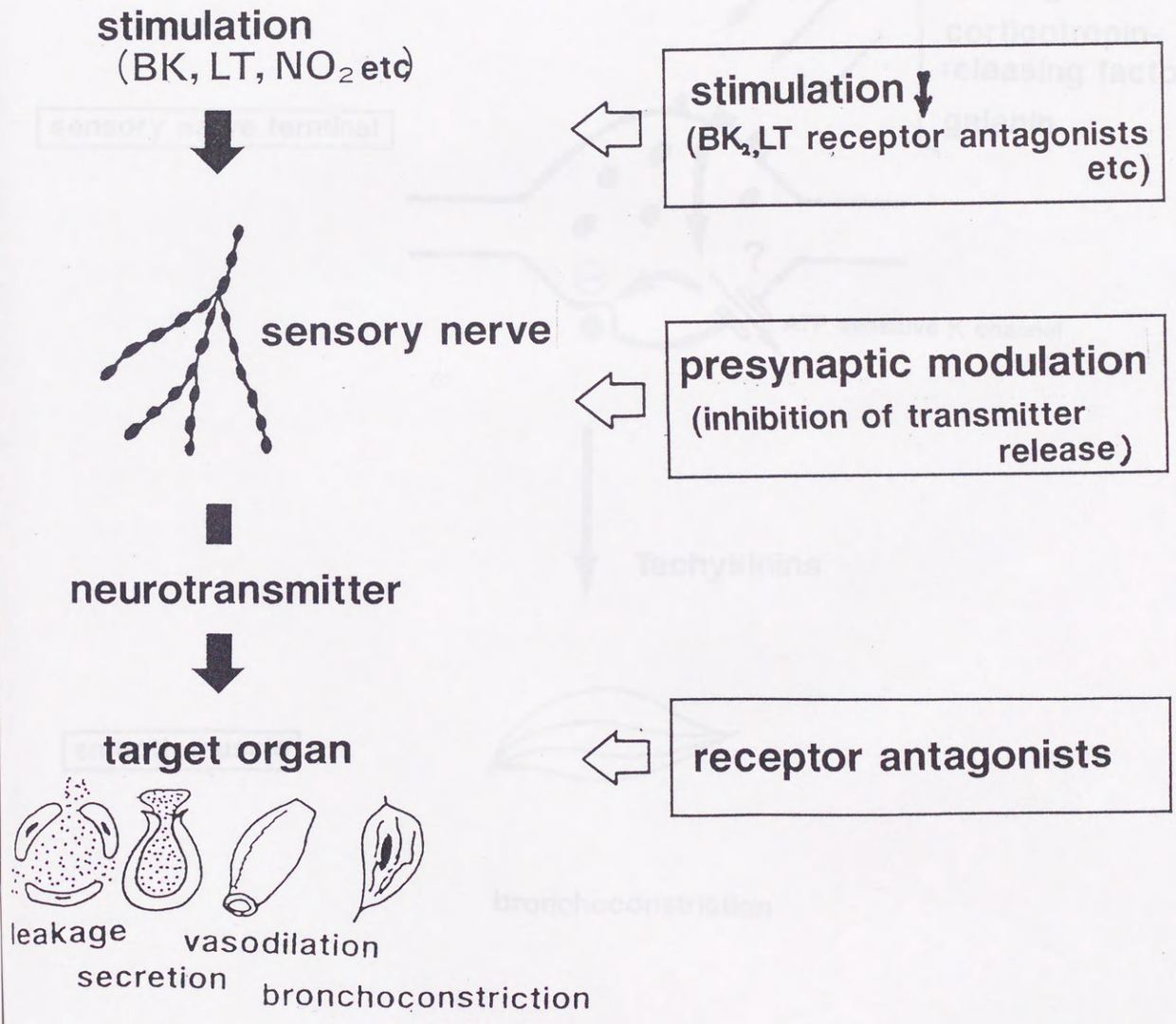


Figure 1 Three possible ways to modulate neurogenic inflammation.

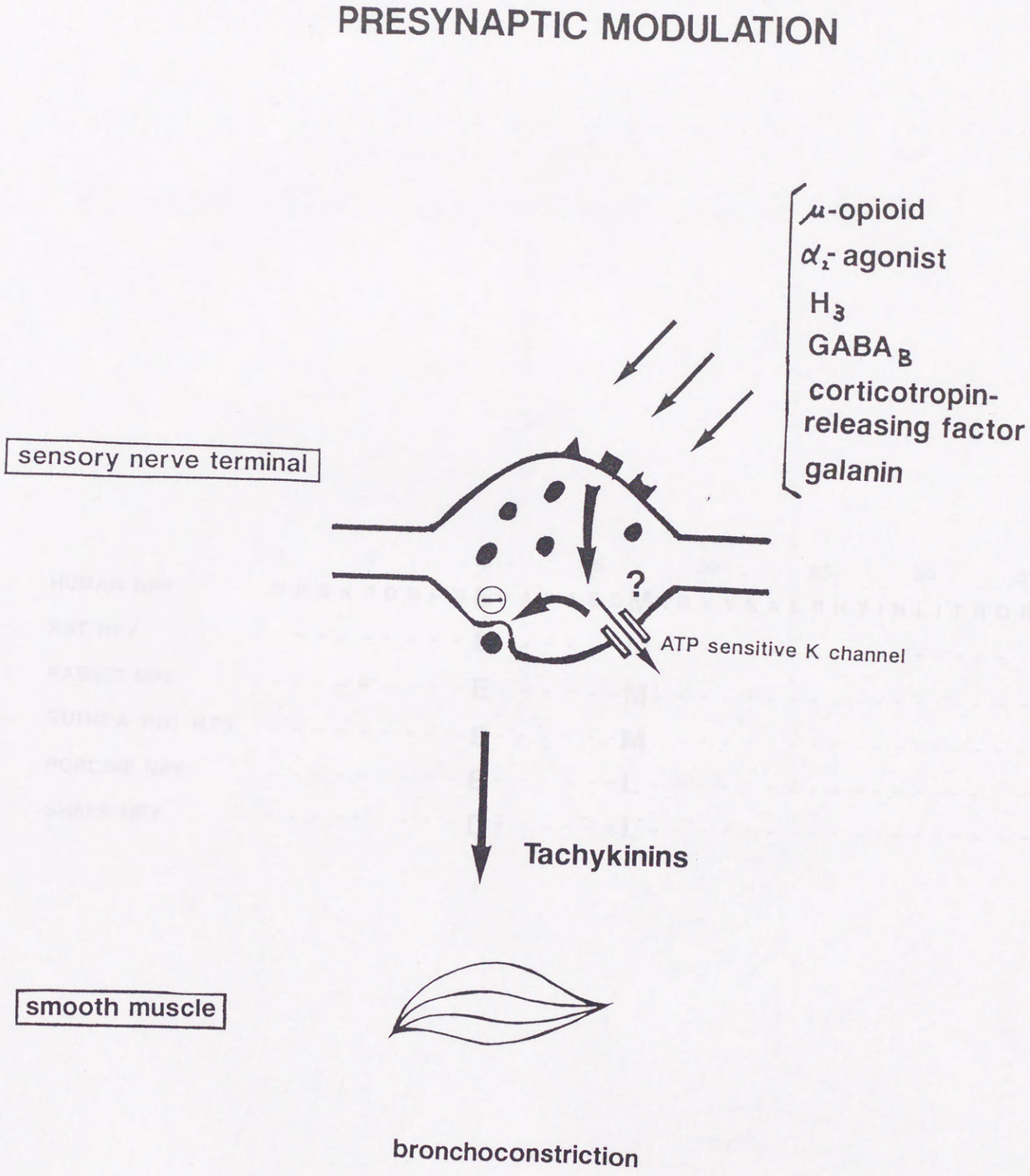


Figure 2 Schematic explanation of presynaptic modulation of sensory nerve-mediated bronchoconstriction. H_3, histamine H_3. $GABA_B$, γ-aminobutylic $acid_B$.

Figure 3

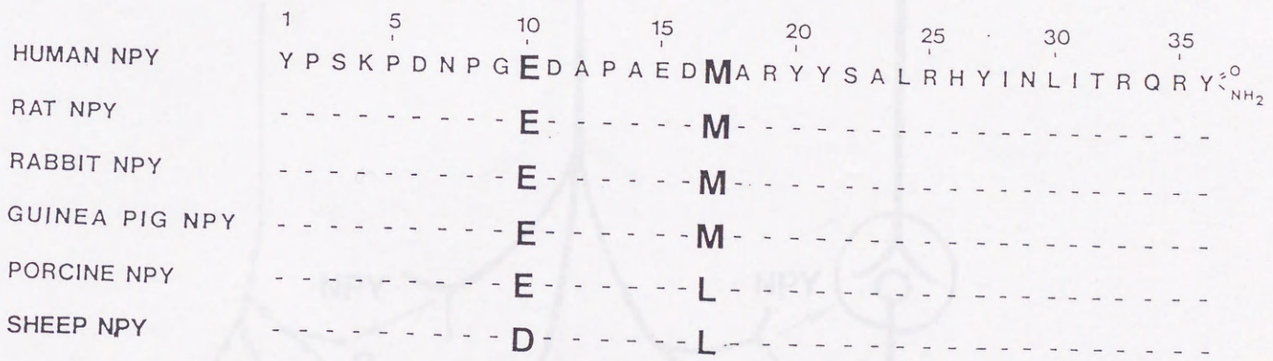


Figure 3 Comparison of the structure of NPYs from different mammalian species. The one-letter code of amino acids has been used. Human NPY has a Glu residue (E) in position 10 and a Met residue (M) in position 17.

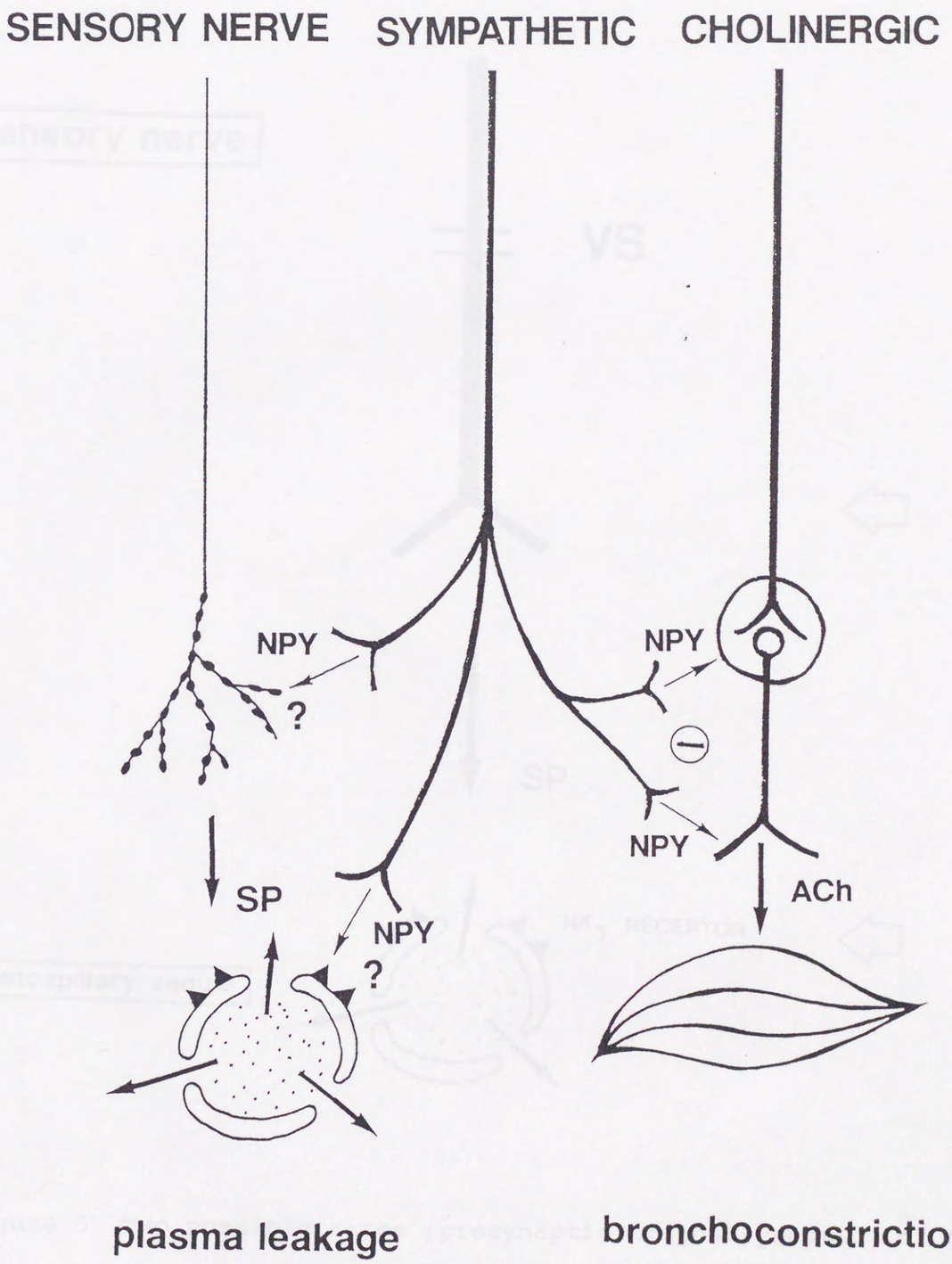


Figure 4 Schematic explanation of the effect of NPY in airways. ACh, acetylcholine. SP, substance P.

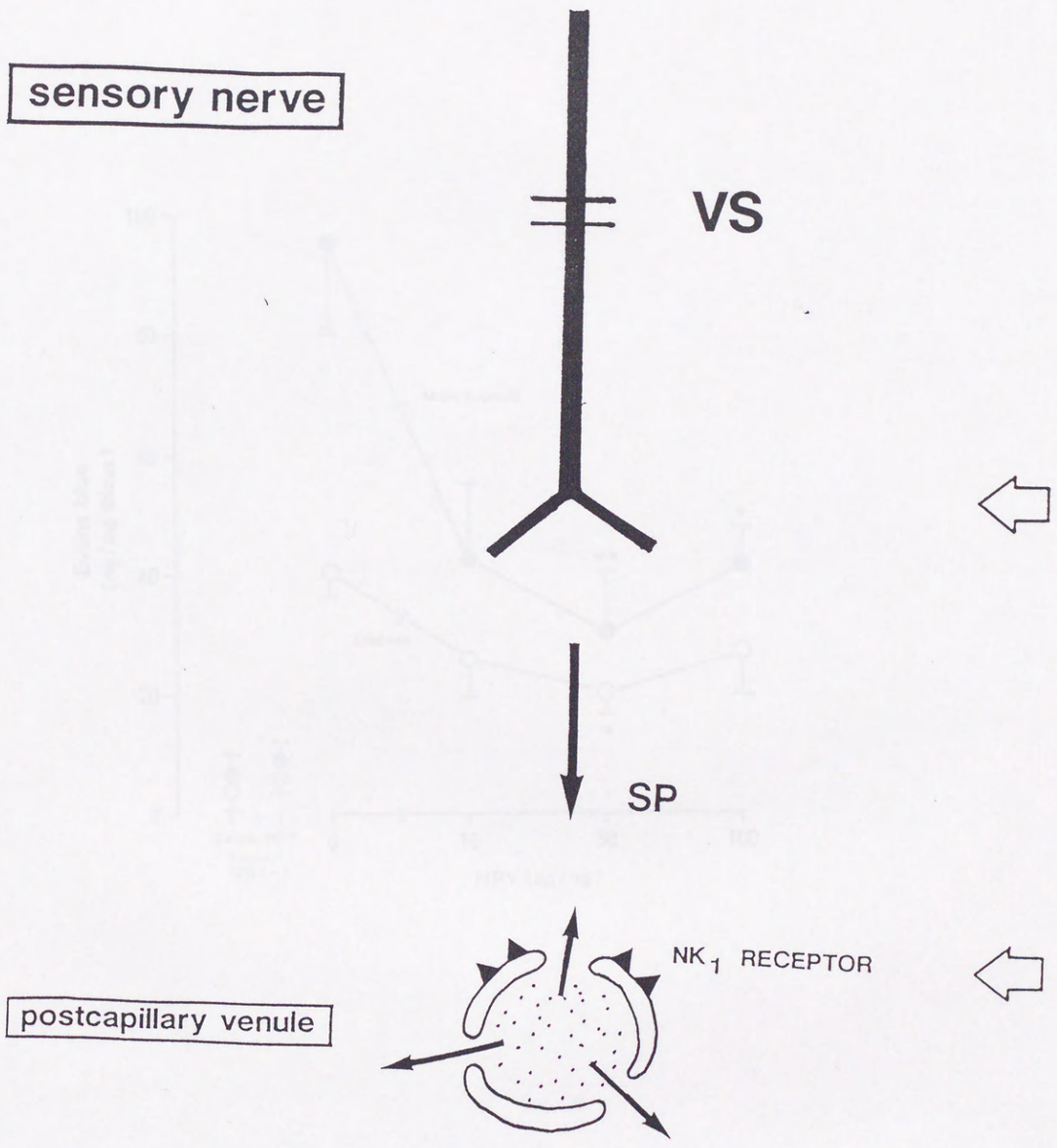


Figure 5 Two possible sites (presynaptic or postsynaptic) of the modulatory action of agonists on sensory nerve-mediated plasma leakage induced by vagal stimulation (VS). SP, substance P.

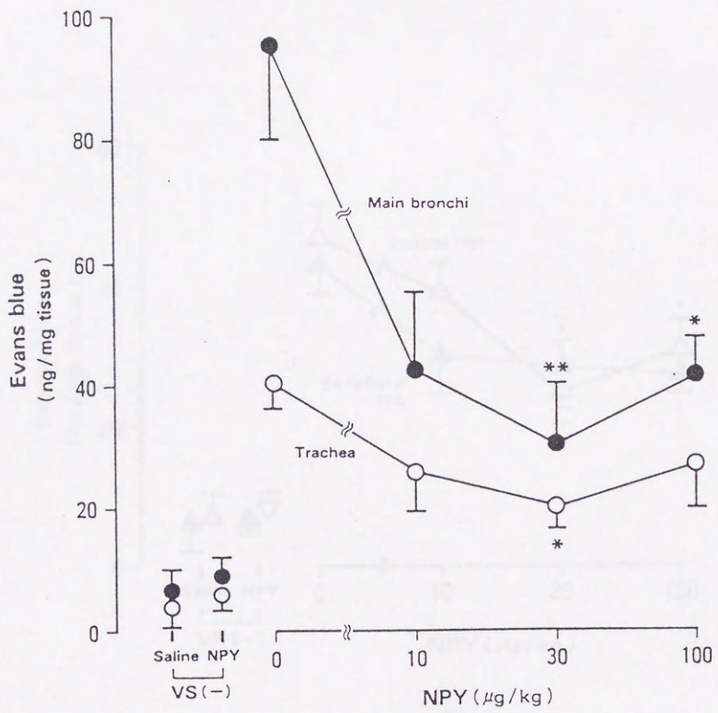


Figure 6 Effects of NPY on sensory nerve-mediated plasma extravasation induced by vagal stimulation (VS) in trachea and main bronchi. Values are means ± SE of 6 animals. Significantly different from saline (vehicle for NPY) values: * $P< 0.05$, ** $P<0.01$ (2-tailed Mann-Whitney U test).

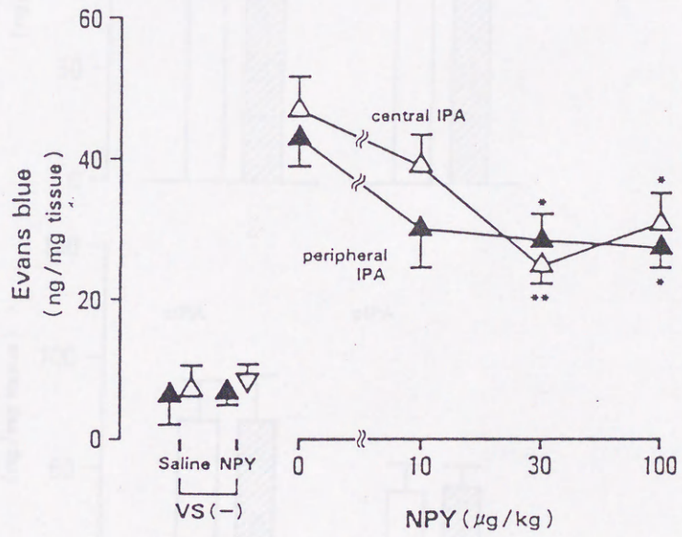


Figure 7 Effects of NPY on sensory nerve-mediated plasma extravasation induced by vagal stimulation (VS) in central and peripheral intrapulmonary airways (IPA). Values are means ± SE of 6 animals. Significantly different from saline values: * $P< 0.05$, ** $P< 0.01$ (2-tailed Mann-Whitney U test).

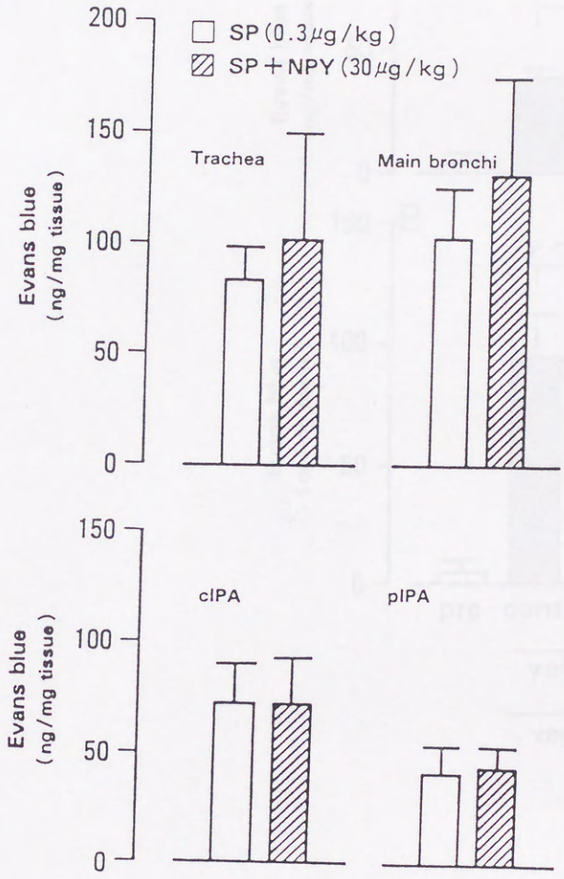


Figure 8 Values are means ± SE of 6 animals expressed as tissue content of Evans blue dye (ng/mg tissue) after intravenous injection of substance P (0.3 μg/kg) after saline (SP) and substance P after NPY (30 μg/kg)(SP + NPY). No significant differences were observed (2-tailed Mann-Whitney *U* test).

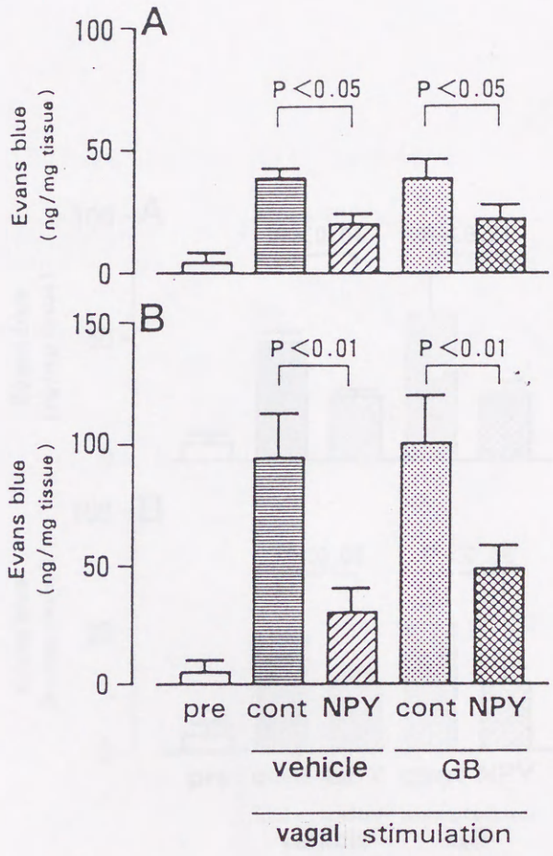


Figure 9 Inhibition of sensory nerve-mediated plasma extravasation by NPY (30 μg/kg iv). Effects of vehicle for glibenclamide (glucose, NaOH and H_2O) and glibenclamide (GB, 25 mg/kg iv) on inhibitory effect of NPY are shown. A: trachea. B: main bronchi. Data are means ± SE of 6 animals. Differences between means were analyzed by 2-tailed Mann-Whitney *U* test.

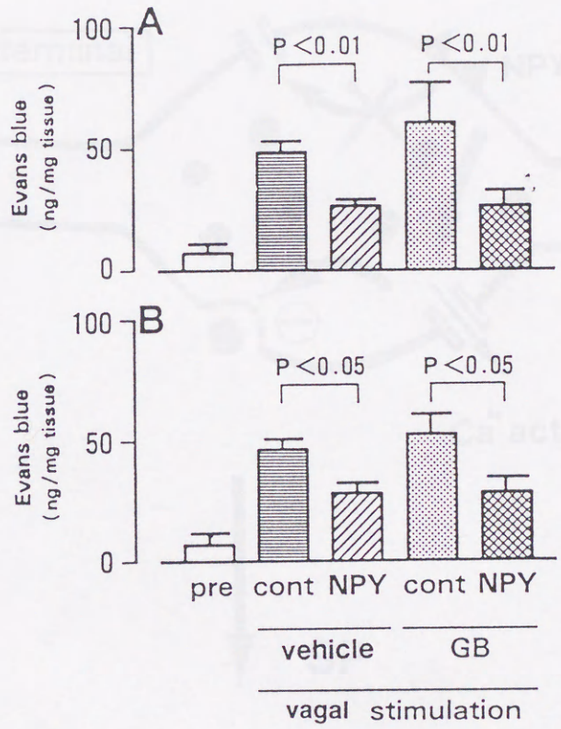


Figure 10 Inhibitory effect of NPY (30μg/kg iv) on sensory nerve-mediated Evans blue dye extravasation in airways. Effects of vehicle for GB and GB (25mg/kg iv) on inhibitory effect of NPY are shown. A: central IPA. B: peripheral IPA. Data are means ±SE of 6 animals. Differences between means were analyzed by 2-tailed Mann-Whitney *U* test.

Figure 11

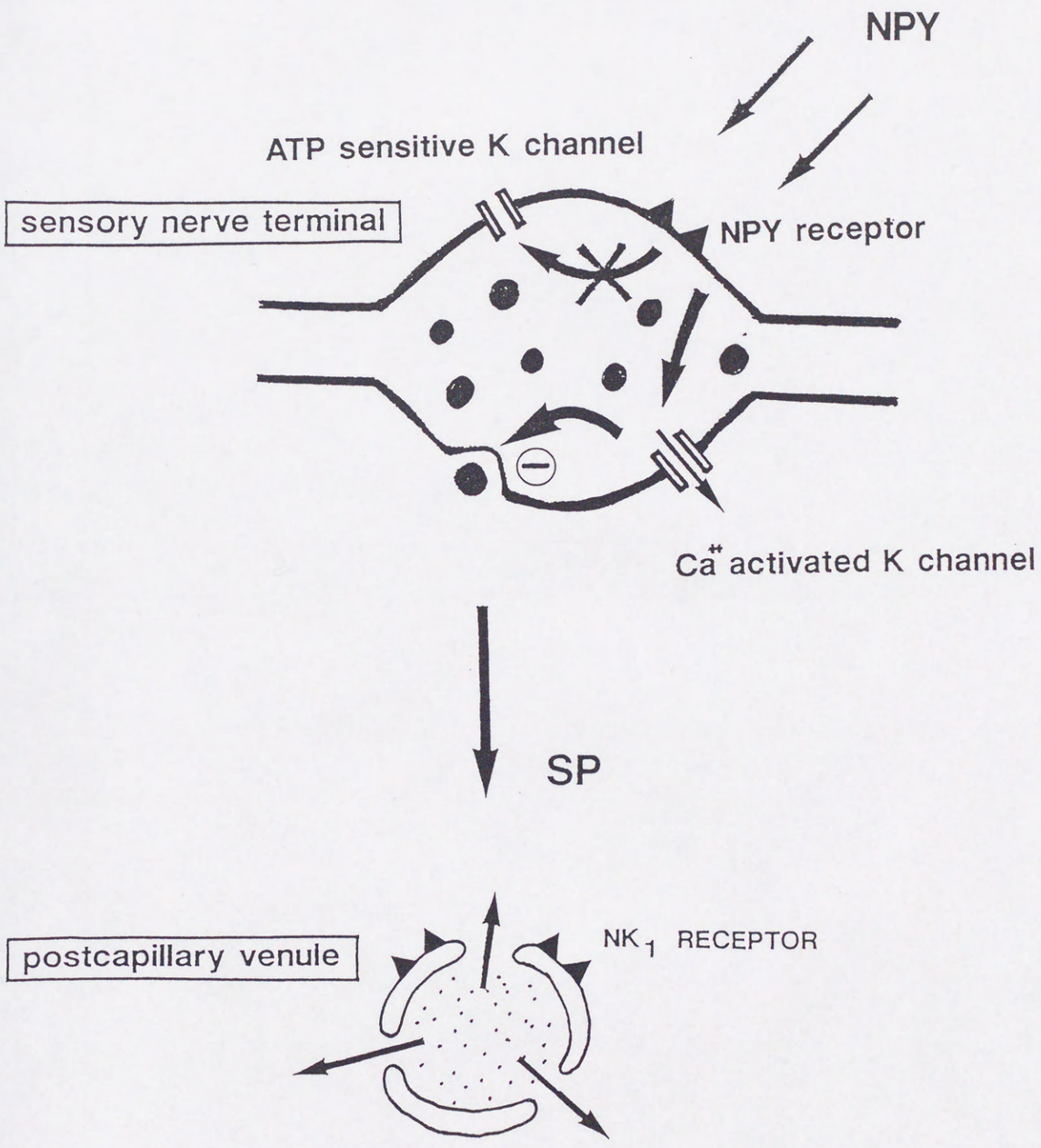
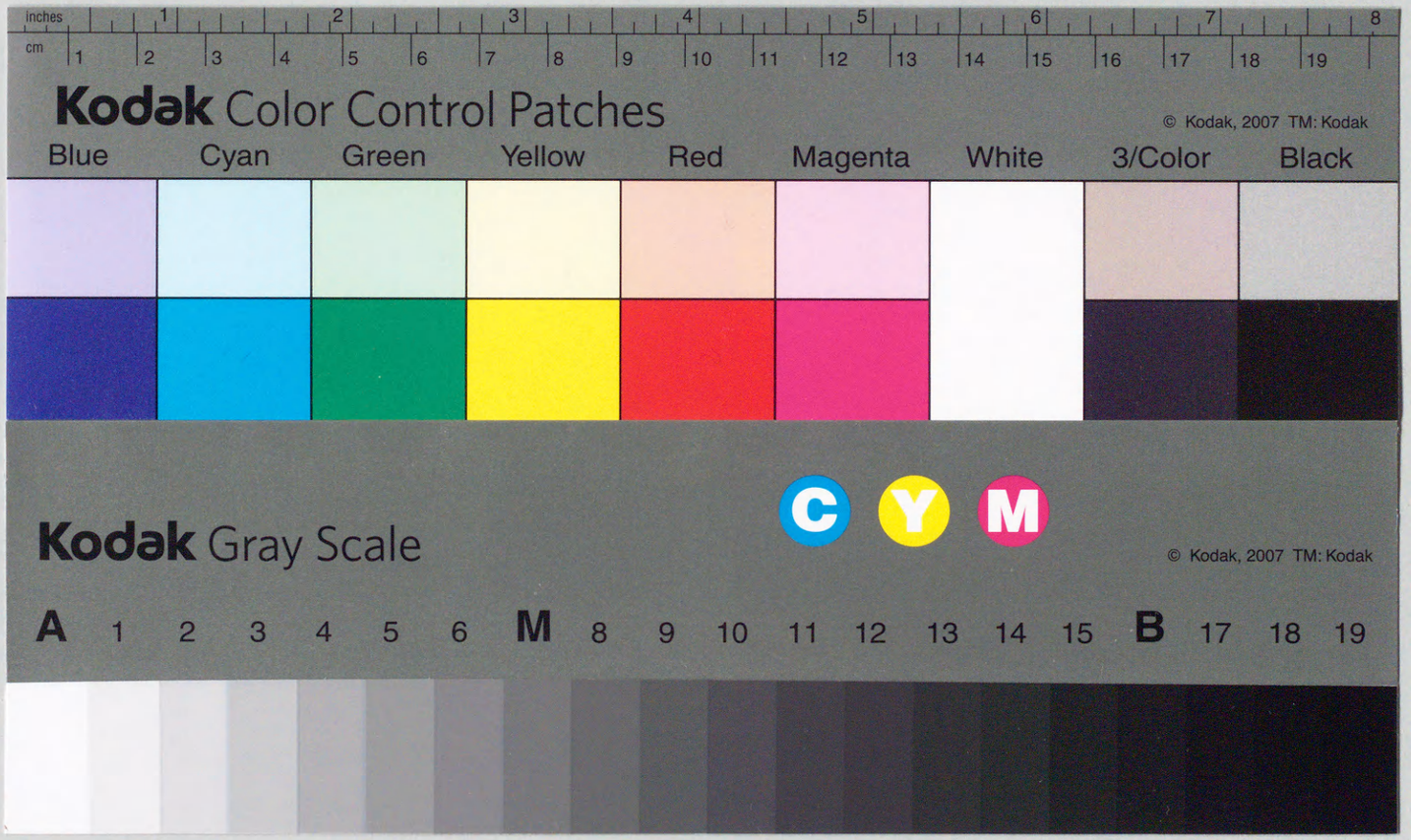


Figure 11 Schematic explanation of possible mechanism of NPY-induced presynaptic modulation of neurogenic inflammation. SP, substance P.


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19